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PATENT SPECIFICATION (11) 1411450

1411450

(21) Application No. 58485/72 (22) Filed 19 Dec. 1972
(23) Complete Specification filed 11 Dec. 1973
(44) Complete Specification published 22 Oct. 1975
(51) INT CL² C12C 11/24
(52) Index at acceptance
C6F 1E 2E

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(19)

(54) PROCESS FOR THE PURIFICATION OF A MICRO-ORGANISM PRODUCT

(71) We, THE BRITISH PETROLEUM COMPANY LIMITED, of Britannic House, Moor Lane, London, EC2Y 9BU, a British Company, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed to be particularly described in and by the following statement:—

The present invention relates to a process for the purification of a micro-organism product obtained by cultivating a hydrocarbon utilising strain of a micro-organism for example a yeast, on a hydrocarbon substrate in the presence of an aqueous nutrient medium and an oxygen containing gas. In particular the invention relates to a process for the removal of lipids and residual hydrocarbons from the micro-organism product.

The micro-organism product recovered from hydrocarbon fermentations is contaminated with residual hydrocarbons. Furthermore it has been found that yeasts cultivated on hydrocarbon substrates, e.g. petroleum fractions have a relatively high lipid content in comparison with yeasts grown on the more conventional carbon, hydrogen and oxygen containing compounds as substrates.

Processes have been proposed for the purification of this microorganism product which involve a solvent extraction treatment whereby the lipid and/or hydrocarbon content is reduced or eliminated. The object of these purification processes is a provide a micro-organism material which is suitable for use as a foodstuff.

Typically in such processes a micro-organism product either dry or in the form of a cream or paste containing water is subjected to a wash or more usually a series of washes with an organic solvent e.g. an alcohol or a mixture of alcohol and hydrocarbon, until a material of the desired purity is obtained.

When the micro-organism product consists essentially of single cell micro-organisms, e.g. a yeast, we have found that complex apparatus is required for satisfactory solvent extraction, particularly where, as is usually the case, the micro-organism product is in the form of a powder having a very small particle size. Constraints are set on the type of solvent extraction apparatus which will provide efficient extraction due to the long sedimentation times encountered with product having a very small particle size. Generally in such a case we have found it necessary to employ multi-stage countercurrent extraction incorporating intermediate forced separation stages so as to obtain a material having the required purity.

In commercial scale operation it is highly desirable, if not essential for economic reasons alone, to provide a plant in which relatively simple and preferably conventional types of apparatus can be used in the solvent extraction stages for the purification of the micro-organism product.

It is an object of the present invention to provide an improved purification process for the removal of substances for example hydrocarbons or lipids from a micro-organism product which is suitable for use in relatively simple and preferably conventional type of apparatus, for example a simple cylinder with a once through extraction. We have now discovered that the difficulties previously mentioned can be overcome by forming the micro-organism product into granules having a specific particle size prior to extraction with solvents.

Accordingly the present invention is a process for the purification of a micro-organism product obtained by cultivating a hydrocarbon utilising strain of a micro-organism on a hydrocarbon substrate in the presence of an aqueous nutrient medium and an oxygen containing gas, the process comprising extracting the micro-organism product in the form of granules having an average particle size in the range 50 to 500 microns with an organic solvent for hydrocarbons and/or lipids to give a micro-

[Price 33p]

organism material having a reduced hydrocarbon and/or lipid content or which is free from hydrocarbons and/or lipids.

The process can be carried out on a commercial scale in conventional simple single stage extractors of the "Olier" or "Lodige" type. The "Olier" type extractor is commonly used in the refining of sugar.

The granulated micro-organism product can be subjected to a heat treatment before extraction with the solvent. The temperature for the heat treatment can be in the range 100°C to 140°C. The time of heat treatment can be in the range 15 to 45 minutes. It is convenient to use a temperature of about 120°C for about 30 minutes. In some cases such a treatment was found to improve the extraction process.

In practice the granules usually have an average particle size in the range 100 to 500 microns. Most suitably the average particle size is in the range 100 to 200 microns and preferably is about 100 microns.

The solvent extraction can be carried out at a temperature in the range 60°C to 80°C and preferably 70° to 75°C. The contact time of solvent and granulated micro-organism product can be in the range 30 to 60 minutes and preferably 40 to 50 minutes.

In the extraction operation allowed the solvent can be to percolate through the impure granulated micro-organism product under the influence of gravity. This technique can be operated in simple equipment without the use of a pump which would otherwise be required to force the solvent through the material to be extracted. A suitable apparatus is then known type of horizontal — basket extractor which consists of a rotatable drum divided by partitions in a vertical plane into a series of compartments. A solvent feed system is provided at the top of each compartment and a perforated hinged plate forms the base of each compartment. The plate can be opened to discharge the extracted material.

Alternatively the granulated micro-organism product can be moved counter currently to the direction of the solvent feed. A suitable apparatus is a screw conveyor extractor wherein the granulated micro-organism product is moved counter currently to the solvent which is gravity fed.

Some examples of suitable organic solvents are alcohols, ketones, hydrocarbons e.g. hexane or petroleum ether, or chlorinated hydrocarbons.

Typically the alcohols and ketones which can be used in the process are those which have been described in the literature in relation to the known processes for the purification of micro-organism containing material. For example British Patent number 1,049,065 discloses the use of ethyl alcohol, isopropanol and acetone. Other examples of useful alcohols are methyl and butyl alcohol.

Water can be added with the solvent or it can be present, at least in part, in the granulated micro-organism product. It is preferred to carry out the extraction treatment in the presence of between 5 and 15 percent by weight of water in relation to the weight of solvent. The preferred solvent is isopropanol. In practice the maximum weight of water in relation to the weight of solvent present is about 25 percent. Normally less than 20 percent of water is used.

Where the granulated micro-organism product is substantially free from water it is preferred to treat it with a mixture containing the desired amount of water and solvent. For example it is often convenient to use an azeotropic mixture of isopropanol and water (i.e. in a proportion by weight of 88 percent isopropanol to 12 percent by weight of water). On the other hand the granulated micro-organism product itself may contain at least a proportion of water, in which case the appropriate proportion of substantially anhydrous solvent may be used.

The micro-organism product can be formed into granules having the desired particle size in the following manner. An aqueous cream containing the micro-organism can be dried using conventional methods such as spray drying to form a powder. The powder thus formed can be mixed with sufficient water to form a granulated mixture and the mixture sifted to recover granules having the desired particle size.

Alternatively an aqueous cream of paste containing the micro-organism, residual hydrocarbon and spent aqueous nutrient medium can be dried to give a powder containing up to about 40 percent by weight of water on the dry weight of the micro-organism. Suitable drying techniques include spray drying, drum drying or evaporation using a conventional evaporator, or a fluid bed dryer.

The powder thus formed is then rehydrated if the water content is below 20 percent by weight to give a paste having a water content in the range of about 20 to 40 percent by weight on the dry weight of the micro-organism. The paste thus formed is further dried to a water content of about 5 percent by weight. Conveniently rehydration can be carried out by mixing the powder with the original cream or paste containing the micro-organism.

The partially dried paste containing the mirco-organism is then sifted, for example by passage through a sieve, to recover granules having the desired particle size.

Alternatively the powder can be compressed to form the granules.

The preferred micro-organisms are single celled, for example hydrocarbon utilising yeasts and in particular members of the genus Candida, e.g. *C. lipolytica* and *C. tropicalis*.

The purified micro-organism material of the present process which has been freed from the whole or part of the contaminant hydrocarbons and/or lipids in accordance with the process hereinbefore described has an improved taste and is valuable foodstuff.

The lipids removed from the micro-organism product can be used as oil additives, for leather fat liquoring and in addition they form a useful source of fatty acids.

The process of the present invention is further illustrate by but not limited to the following Examples.

Example 1

A hydrocarbon utilising strain of the yeast, *Candida tropicalis*, was grown in aerated conditions using a heavy gas oil as the carbon substrate and an aqueous solution of nutrient mineral salts. A cream consisting essentially or about 14.7 percent by weight of *Candida tropicalis*, 0.3 percent by weight of residual gas oil and 85 percent by weight of spent aqueous nutrient medium. was recovered from the cultivated broth by decantation followed by centrifugation. This cream was passed to a spray drier and dried to give a yeast product in the form of a powder having a water content of about 5 percent by weight. The yeast at this stage had a lipid content of about 13 percent by weight (estimated on the dry weight of the yeast). The powder was mixed with the original cream in a proportion of 59.4 percent by weight of powder to 40.6 percent by weight of cream to give a paste containing 52.6 percent by weight of dry yeast, 2.0 percent by weight of residual gas oil, 7.9 percent by weight of lipids and 37.5 percent by weight of water. The paste was passed to a flash dryer where water was removed to give a partially dried product having a water content of about 5 percent by weight in relation to the dry weight of the yeast. The partially dried paste was then passed to a sieve where it was sifted to recover a granulated yeast product having an average particle size of about 315 microns.

The granulated yeast product thus formed was fed to a Lödige extractor wherein the yeast granules are moved slowly by an Archimedes screw in the solvent in a ratio of one part by weight of granulated yeast to 10 parts by weight of an azeoptropic solvent mixture containing 88 percent by weight of isopropanol and 12 percent by weight of water. The yeast was extracted at a temperature of 70°C with one volume of solvent mixture to one volume of granulated yeast. The solvent flow rate was adjusted to give a drainage time of 10 minutes. The extraction was repeated three times after which a yeast material was obtained which was free from residual gas oil and having a total lipid content on a dry weight basis of 1.2 percent.

Example 2

The solvent extraction procedure described in Example 1 was followed using granulated yeast having the following average particle sizes:— 100 microns, 150 microns, 257 microns and 407 microns. The yeast material obtained after solvent extraction was free from residual gas oil and had the following total lipid contents (percent dry weight) respectively:— 1.03, 1.10, 1.20 and 2.3.

Experiment by way of comparison

The solvent extraction procedure described in Example 1 was followed using (a) a spray dried yeast powder having an average particle size of less than 50 microns and (b) a granulated yeast having an average particle size of 565 microns. In the case of the yeast powder (a) it was not possible to obtain an extracted material because the solvent did not pass through the powder. In the latter case the level of residual lipid and hydrocarbon was unacceptably high. The lipid content of the extracted granulated yeast material (b) was 3.8 percent by weight. The material still contained residual hydrocarbon.

WHAT WE CLAIM IS:—

1. A process for the purification of a micro-organism product obtained by cultivating a hydrocarbon utilising strain of a micro-organism on a hydrocarbon substrate in the presence of an aqueous nutrient medium and an oxygen containing gas, the process comprising extracting the micro-organism product in the form of granules having an average particle size in the range 50 to 500 microns with an organic solvent for hydrocarbons and/or lipids to give a micro-organism material having a reduced hydrocarbon and/or lipid content or which is free from hydrocarbons and/or lipids.

2. A process as claimed in claim 1 wherein the granules have a particle size in the range 100 to 200 microns.

3. A process as claimed in claim 1 or 2 wherein extraction is carried out by allowing the solvent to precolate through the granulated micro-organism product under the influence of gravity.

4. A process as claimed in any one of the preceding claims wherein extraction is carried out in the presence of between 5 and 15 percent by weight of water in relation to the weight of solvent.

5. A process as claimed in any one of the preceding claims wherein the solvent is isopropanol.

6. A process as claimed in and one of the preceding claims wherein the extraction is

carried out at a temperature in the range 60°C to 80°C.

7. A process as claimed in any one of the preceding claims wherein the granulated micro-organism product is subjected to a heat treatment at a temperature in the range 100°C to 140°C before extraction with the solvent.

8. A process as claimed in claim 7 wherein the time of heat treatment is in the range 15 to 45 minutes.

9. A process as claimed in any one of the preceding claims wherein the micro-organism is a yeast.

10. A process as claimed in claim 1 and as hereinbefore described with reference to Example 1.

11. A process as claimed in claim 1 and as hereinbefore described with reference to Example 2.

12. A purified micro-organism material when obtained by the process as claimed in any one of the preceding claims.

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Printed for Her Majesty's Stationery Office, by the Courier Press, Leamington Spa, 1975.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.